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(54) Title: FLUORESCENT PROTEINS FROM NON-BIOLUMINESCENT SPECIES OF CLASS ANTHOZOA, GENES ENCODING SUCH PROTEINS AND USES THEREOF		
(57) Abstract		
The present invention is directed to novel fluorescent proteins from non-bioluminescent organisms from the Class Anthozoa. Also disclosed are methods of identifying nucleic acid sequence encoding the fluorescent proteins and further analyzing the proteins.		

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**FLUORESCENT PROTEINS FROM NON-BIOLUMINESCENT SPECIES
OF CLASS ANTHOZOA, GENES ENCODING SUCH PROTEINS AND
USES THEREOF**

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BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to the field of molecular biology. More specifically, this invention relates to novel fluorescent proteins, 15 methods of identifying the DNA sequences encoding the proteins and uses thereof.

Description of the Related Art

Fluorescence labeling is a particularly useful tool for marking a protein, cell, or organism of interest. Traditionally, a protein of interest is purified, then covalently conjugated to a fluorophore derivative. For *in vivo* studies, the protein-dye complex is then inserted into cells of interest using micropipetting or a method of reversible permeabilization. The dye attachment and insertion steps, 25 however, make the process laborious and difficult to control. An alternative method of labeling proteins of interest is to concatenate or fuse the gene expressing the protein of interest to a gene expressing a marker, then express the fusion product. Typical markers for this method of protein labeling include β -galactosidase, firefly luciferase

and bacterial luciferase. These markers, however, require exogenous substrates or cofactors and are therefore of limited use for *in vivo* studies.

A marker that does not require an exogenous cofactor or
5 substrate is the green fluorescent protein (GFP) of the jellyfish *Aequorea victoria*, a protein with an excitation maximum at 395 nm, a second excitation peak at 475 nm and an emission maximum at 510 nm. GFP is a 238-amino acid protein, with amino acids 65-67 involved in the formation of the chromophore.

10 Uses of GFP for the study of gene expression and protein localization are discussed in detail by Chalfie et al. in *Science* 263 (1994), 802-805, and Heim et al. in *Proc. Nat. Acad. Sci.* 91 (1994), 12501-12504. Additionally, Rizzuto et al. in *Curr. Biology* 5 (1995), 635-642, discuss the use of wild-type GFP as a tool for visualizing
15 subcellular organelles in cells, while Kaether and Gerdes in *Febs Letters* 369 (1995), 267-271, report the visualization of protein transport along the secretory pathway using wild-type GFP. The expression of GFP in plant cells is discussed by Hu and Cheng in *Febs Letters* 369 (1995), 331-334, while GFP expression in *Drosophila* embryos is described by
20 Davis et al. in *Dev. Biology* 170 (1995), 726-729.

Crystallographic structures of wild-type GFP and the mutant GFP S65T reveal that the GFP tertiary structure resembles a barrel (Ormö et al., *Science* 273 (1996), 1392-1395; Yang, et al., *Nature Biotechnol* 14 (1996), 1246-1251). The barrel consists of beta sheets
25 in a compact structure, where, in the center, an alpha helix containing the chromophore is shielded by the barrel. The compact structure makes GFP very stable under diverse and/or harsh conditions such as protease treatment, making GFP an extremely useful reporter in

general. However, the stability of GFP makes it sub-optimal for determining short-term or repetitive events.

A great deal of research is being performed to improve the properties of GFP and to produce GFP reagents useful and optimized for 5 a variety of research purposes. New versions of GFP have been developed, such as a "humanized" GFP DNA, the protein product of which has increased synthesis in mammalian cells (Haas, et al., *Current Biology* 6 (1996), 315-324; Yang, et al., *Nucleic Acids Research* 24 (1996), 4592-4593). One such humanized protein is "enhanced green 10 fluorescent protein" (EGFP). Other mutations to GFP have resulted in blue-, cyan- and yellow-green light emitting versions. Despite the great utility of GFP, however, other fluorescent proteins with properties similar to or different from GFP would be useful in the art. Novel 15 fluorescent proteins result in possible new colors, or produce pH-dependent fluorescence. Other benefits of novel fluorescent proteins include fluorescence resonance energy transfer (FRET) possibilities based on new spectra and better suitability for larger excitation.

The prior art is deficient in novel fluorescent proteins wherein the DNA coding sequences are known. The present invention 20 fulfills this long-standing need in the art.

SUMMARY OF THE INVENTION

25 The present invention is directed to an isolated and purified fluorescent protein selected from the group consisting of amFP486, cFP484, zFP506, zFP538, dsFP483, drFP583, asFP600, dgFP512 and dmFP592.

In one embodiment of the present invention, there is provided a method of identifying a DNA sequence encoding a fluorescent protein comprising the step of screening for an existence of a nucleic acid sequence in a sample, wherein the nucleic acid sequence encodes a peptide having a sequence selected from the group consisting of SEQ ID Nos. 3, 5, 8, 11, 12 and 14. The existence of the nucleic acid sequence identifies the DNA sequence encoding the fluorescent protein.

In another embodiment of the present invention, there is provided a method of identifying a DNA sequence encoding a fluorescent protein comprising the step of screening for an existence of a nucleic acid sequence in a sample, wherein the nucleic acid sequence hybridizes to a primer selected from the group consisting of SEQ ID Nos. 4, 6, 7, 9, 10, 13, 15 and 16. The existence of the nucleic acid sequence identifies the DNA sequence encoding the fluorescent protein.

In still another embodiment of the present invention, there is provided a method of analyzing a fluorescent protein in a cell, comprising the steps of expressing a nucleic acid sequence encoding a fluorescent protein having an amino acid sequence selected from the group consisting of SEQ ID Nos. 55-63 in the cell; and measuring a fluorescence signal from the protein. This method further comprises a step of sorting the cell according to the signal. Preferably, the cell is sorted by fluorescence activated cell sorting. Still preferably, the nucleic acid sequence comprises a gene of interest encoding a protein of interest fused to the fluorescent protein, wherein the protein of interest is distinct from the fluorescent protein. The detected fluorescence signal indicates the presence of the gene of interest and further the protein of interest in the cell. By identifying an

intracellular location of the fluorescent protein, an intracellular location of the protein of interest is also identified.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of 5 the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figure 1** shows the modified strategy of 3'-RACE used to isolate the target fragments. Sequences of the oligonucleotides used are shown in Table 2. Dp1 and Dp2 are the degenerate primers used in the first and second PCR, respectively (see Tables 3 and 4 for the sequences of degenerate primers).

15 **Figure 2A** shows multiple alignment of novel fluorescent proteins. The numbering is based on *Aequorea victoria* green fluorescent protein (GFP). Two proteins from *Zoanthus* and four from *Discosoma* are compared between each other: residues identical to the corresponding ones in the first protein of the series are represented by 20 dashes. Introduced gaps are represented by dots. In the sequence of *A. victoria* GFP, the stretches forming beta-sheets are underlined; the residues whose side chains form the interior of the beta-can are shaded (according to Yang et al., *Nature Biotechnol.* 14, 1246–1251 (1996)).
25 **Figure 2B** shows the N-terminal part of cFP484, which has no homology with the other proteins. The putative signal peptide is underlined.

Figure 3 shows the excitation and emission spectrum of the novel fluorescent protein from *Anemonia majano*, amFP486.

Figure 4 shows the excitation and emission spectrum of the novel fluorescent protein from *Clavularia*, cFP484.

Figure 5 shows the excitation and emission spectrum of the novel fluorescent protein from *Zoanthus*, zFP506.

5 Figure 6 shows the excitation and emission spectrum of the novel fluorescent protein from *Zoanthus*, zFP538.

Figure 7 shows the excitation and emission spectrum of the novel fluorescent protein from *Discosoma striata*, dsFP483.

10 Figure 8 shows the excitation and emission spectrum of the novel fluorescent protein from *Discosoma*, drFP583.

Figure 9 shows the excitation and emission spectrum of the novel fluorescent protein from *Anemonia sulcata*, asFP600.

Figure 10 shows the excitation and emission spectrum of the novel fluorescent protein from *Discosoma*, dgFP512.

15 Figure 11 shows the excitation and emission spectrum of the novel fluorescent protein from *Discosoma*, dmFP592.

DETAILED DESCRIPTION OF THE INVENTION

20 As used herein, the term "GFP" refers to the basic green fluorescent protein from *Aequorea victoria*, including prior art versions of GFP engineered to provide greater fluorescence or fluoresce in different colors. The sequence of *Aequorea victoria* GFP (SEQ ID No. 54) has been disclosed in Prasher et al., *Gene* 111 (1992), 229-33

25 As used herein, the term "EGFP" refers to mutant variant of GFP having two amino acid substitutions: F64L and S65T (Heim et al., *Nature* 373 (1995), 663-664). The term "humanized" refers to changes made to the GFP nucleic acid sequence to optimize the codons for

expression of the protein in human cells (Yang et al., *Nucleic Acids Research* 24 (1996), 4592-4593).

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" (B.D. Hames & S.J. Higgins eds. (1985)); "Transcription and Translation" (B.D. Hames & S.J. Higgins eds. (1984)); "Animal Cell Culture" (R.I. Freshney, ed. (1986)); "Immobilized Cells and Enzymes" (IRL Press, (1986)); B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in either single stranded form or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes.

A DNA "coding sequence" is a DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3'

(carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and synthetic DNA sequences. A polyadenylation signal and transcription termination 5 sequence may be located 3' to the coding sequence.

As used herein, the term "hybridization" refers to the process of association of two nucleic acid strands to form an antiparallel duplex stabilized by means of hydrogen bonding between residues of the opposite nucleic acid strands.

10 The term "oligonucleotide" refers to a short (under 100 bases in length) nucleic acid molecule.

"DNA regulatory sequences", as used herein, are transcriptional and translational control sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that 15 provide for and/or regulate expression of a coding sequence in a host cell.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining 20 the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a 25 transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Various promoters, including inducible promoters, may be used to drive the various vectors of the present invention.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" or "transfected" by 5 exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to 10 eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter 15 cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

A "heterologous" region of the DNA construct is an 20 identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, 25 heterologous DNA includes coding sequence in a construct where portions of genes from two different sources have been brought together so as to produce a fusion protein product. Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

As used herein, the term "reporter gene" refers to a coding sequence attached to heterologous promoter or enhancer elements and whose product may be assayed easily and quantifiably when the construct is introduced into tissues or cells.

5 The amino acids described herein are preferred to be in the "L" isomeric form. The amino acid sequences are given in one-letter code (A: alanine; C: cysteine; D: aspartic acid; E: glutamic acid; F: phenylalanine; G: glycine; H: histidine; I: isoleucine; K: lysine; L: leucine; M: methionine; N: asparagine; P: proline; Q: glutamine; R: arginine; S: 10 serine; T: threonine; V: valine; W: tryptophane; Y: tyrosine; X: any residue). NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243 (1969), 3552-15 59 is used.

The present invention is directed to an isolated and purified fluorescent protein selected from the group consisting of amFP486, cFP484, zFP506, zFP538, dsFP483, drFP583, asFP600, dgFP512 and dmFP592.

20 In one embodiment of the present invention, there is provided a method of identifying a DNA sequence encoding a fluorescent protein comprising the step of screening for an existence of a nucleic acid sequence in a sample, wherein the nucleic acid sequence encodes a peptide having a sequence selected from the group 25 consisting of SEQ ID Nos. 3, 5, 8, 11, 12 and 14. The existence of the nucleic acid sequence identifies the DNA sequence encoding the fluorescent protein.

In another embodiment of the present invention, there is provided a method of identifying a DNA sequence encoding a

fluorescent protein comprising the step of screening for an existence of a nucleic acid sequence in a sample, wherein the nucleic acid sequence hybridizes to a primer selected from the group consisting of SEQ ID Nos. 4, 6, 7, 9, 10, 13, 15 and 16. The existence of the nucleic acid 5 sequence identifies the DNA sequence encoding the fluorescent protein.

In still another embodiment of the present invention, there is provided a method of analyzing a fluorescent protein in a cell, comprising the steps of expressing a nucleic acid sequence encoding a 10 fluorescent protein having an amino acid sequence selected from the group consisting of SEQ ID Nos. 55-63 in the cell; and measuring a fluorescence signal from the protein. This method further comprises a step of sorting the cell according to the signal. Preferably, the cell is sorted by fluorescence activated cell sorting. Still preferably, the 15 nucleic acid sequence comprises a gene of interest encoding a protein of interest fused to the fluorescent protein, wherein the protein of interest is distinct from the fluorescent protein. The detected fluorescence signal indicates the presence of the gene of interest and further the protein of interest in the cell. By identifying an 20 intracellular location of the fluorescent protein, an intracellular location of the protein of interest is also identified.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1**Biological Material**

5 Novel fluorescent proteins were identified from several genera of Anthozoa which do not exhibit any bioluminescence but have fluorescent color as observed under usual white light or ultraviolet light. Six species were chosen (see Table 1).

10

TABLE 1**Anthozoa Species Used in This Study**

Species	Area of Origination	Fluorescent Color
Anemonia majano	Western Pacific	bright green tentacle tips
Clavularia sp.	Western Pacific	bright green tentacles and oral disk
Zoanthus sp.	Western Pacific	green-yellow tentacles and oral disk
Discosoma sp. "red"	Western Pacific	orange-red spots oral disk
Discosoma striata	Western Pacific	blue-green stripes on oral disk
Discosoma sp. "magenta"	Western Pacific	faintly purple oral disk
Discosoma sp.	Western Pacific	green spots on oral disk

"green"		
Anemonia sulcata	Mediterranean	purple tentacle tips

EXAMPLE 2**cDNA Preparation**

Total RNA was isolated from the species of interest
5 according to the protocol of Chomczynski and Sacchi (Chomczynski P., et al., *Anal. Biochem.* 162 (1987), 156-159). First-strand cDNA was synthesized starting with 1-3 µg of total RNA using SMART PCR cDNA synthesis kit (CLONTECH) according to the provided protocol with the only alteration being that the "cDNA synthesis primer" provided in the
10 kit was replaced by the primer TN3 (5'- CGCAGTCGACCG(T)₁₃, SEQ ID No. 1) (Table 2). Amplified cDNA samples were then prepared as described in the protocol provided except the two primers used for PCR were the TS primer (5'-AAGCAGTGGTATCAACGCAGAGT, SEQ ID No. 2) (Table 2) and the TN3 primer (Table 2), both in 0.1 µM concentration.
15 Twenty to twenty-five PCR cycles were performed to amplify a cDNA sample. The amplified cDNA was diluted 20-fold in water and 1 µl of this dilution was used in subsequent procedures.

TABLE 2Oligos Used in cDNA Synthesis and RACE

5 TN3: 5'-CGCAGTCGACCG(T)₁₃
(SEQ ID No. 1)

T7-TN3: 5'-GTAATACGACTCACTATAAGGCCGCAGTCGACCG(T)₁₃
(SEQ ID No. 17)

10 TS-primer: 5'-AAGCAGTGGTATCAACGCAGAGT
(SEQ ID No. 2)

15 T7-TS:
5'-GTAATACGACTCACTATAAGGCAGCAGTGTTATCAACGCAGAGT
(SEQ ID No. 18)

T7: 5'-GTAATACGACTCACTATAAGGC
(SEQ ID No. 19)

20 TS-oligo 5'-AAGCAGTGGTATCAACGCAGAGTACGCrGrGrG
(SEQ ID No. 53)

25

EXAMPLE 3**Oligo Design**

To isolate fragments of novel fluorescent protein cDNAs,
5 PCR using degenerate primers was performed. Degenerate primers
were designed to match the sequence of the mRNAs in regions that
were predicted to be the most invariant in the family of fluorescent
proteins. Four such stretches were chosen (Table 3) and variants of
degenerate primers were designed. All such primers were directed to
10 the 3'-end of mRNA. All oligos were gel-purified before use. Table 2
shows the oligos used in cDNA synthesis and RACE.

TABLE 3

Key Amino Acid Stretches and Corresponding Degenerate Primers Used for Isolation of Fluorescent Proteins

5

Stretch Position according to A. victoria GFP (7)	Amino Acid Sequence of the Key Stretch	Degenerated Primer Name and Sequence
20-25	GXVN GH (SEQ ID No. 3)	NGH: 5'- GA(C,T) GGC TGC GT(A,T,G,C) AA(T,C) GG(A,T,G) CA (SEQ ID No. 4)
31-35	GEGEG (SEQ ID No. 5)	GEGa: 5'- GTT ACA GGT GA(A,G) GG(A,C) GA(A,G) GG (SEQ ID No. 6) GEGb: 5'- GTT ACA GGT GA(A,G) GG(T,G) GA(A,G) GG (SEQ ID No. 7)
	GEGNG (SEQ ID No. 8)	GNGa: 5'- GTT ACA GGT GA(A,G) GG(A,C) AA(C,T) GG (SEQ ID No. 9) GNGb: 5'- GTT ACA GGT GA(A,G) GG(T,G) AA(C,T) GG (SEQ ID No. 10)
127-131	GMNFP (SEQ ID No. 11) GVNFP (SEQ ID No. 12)	NFP: 5' TTC CA(C,T) GGT (G,A)TG AA(C,T) TT(C,T) CC (SEQ ID NO. 13)
134-137	GPVM (SEQ ID No. 14)	PVMa: 5' CCT GCC (G,A)A(C,T) GGT CC(A,T,G,C) GT(A,C) ATG (SEQ ID NO. 15) PVMb: 5' CCT GCC (G,A)A(C,T) GGT CC(A,T,G,C) GT(G,T) ATG (SEQ ID NO. 16)

EXAMPLE 4**Isolation of 3'-cDNA Fragments of nFPs**

The modified strategy of 3'-RACE was used to isolate the target fragments (see Figure 1). The RACE strategy involved two consecutive PCR steps. The first PCR step involved a first degenerate primer (Table 4) and the T7-TN3 primer (SEQ ID No. 17) which has a 3' portion identical to the TN3 primer used for cDNA synthesis (for sequence of T7-TN3, Table 2). The reason for substituting the longer T7-TN3 primer in this PCR step was that background amplification which occurred when using the shorter TN3 primer was suppressed effectively, particularly when the T7-TN3 primer was used at a low concentration (0.1 μ M) (Frohman et al., (1998) *PNAS USA*, 85, 8998-9002). The second PCR step involved the TN3 primer (SEQ ID No. 1, Table 2) and a second degenerate primer (Table 4).

TABLE 4

Combinations of Degenerate Primers for First and Second PCR Resulting in Specific Amplification of 3'-Fragments of nFP cDNA

Species	First Degenerate Primer	Second Degenerate Primer
<i>Anemonia majano</i>	NGH (SEQ ID No. 4)	GNGb (SEQ ID No. 10)
<i>Clavularia</i> sp.	NGH (SEQ ID No. 4)	GEGA (SEQ ID No. 6)
<i>Zoanthus</i> sp.	NGH (SEQ ID No. 4)	GEGA (SEQ ID No. 6)
<i>Discosoma</i> sp. "red"	NGH (SEQ ID No. 4)	GEGA (SEQ ID No. 6), NFP (SEQ ID No. 13) or PVMB (SEQ ID No. 16)
<i>Discosoma striata</i>	NGH (SEQ ID No. 4)	NFP (SEQ ID No. 13)
<i>Anemonia sulcata</i>	NGH (SEQ ID No. 4)	GEGA (SEQ ID No. 6) or NFP (SEQ ID No. 13)

5

The first PCR reaction was performed as follows: 1 µl of 20-fold
 10 dilution of the amplified cDNA sample was added into the reaction mixture containing 1X Advantage KlenTaq Polymerase Mix with provided buffer (CLONTECH), 200 µM dNTPs, 0.3 µM of first degenerate

primer (Table 4) and 0.1 μ M of T7-TN3 (SEQ ID No. 17) primer in a total volume of 20 μ l. The cycling profile was (Hybaid OmniGene Thermocycler, tube control mode): 1 cycle for 95°C, 10 sec.; 55°C, 1 min.; 72°C, 40 sec; 24 cycles for 95°C, 10 sec.; 62°C, 30 sec.; 72°C, 40 sec. The reaction was then diluted 20-fold in water and 1 μ l of this dilution was added to a second PCR reaction, which contained 1X Advantage KlenTaq Polymerase Mix with the buffer provided by the manufacturer (CLONTECH), 200 μ M dNTPs, 0.3 μ M of the second degenerate primer (Table 4) and 0.1 μ M of TN3 primer. The cycling profile was (Hybaid OmniGene Thermocycler, tube control mode): 1 cycle for 95°C, 10 sec.; 55°C (for GEG/GNG or PVM) or 52°C (for NFP), 1 min.; 72°C, 40 sec; 13 cycles for 95°C, 10sec.; 62°C (for GEG/GNG or PVM) or 58°C (for NFP), 30 sec.; 72°C, 40 sec. The product of PCR was cloned into PCR-Script vector (Stratagene) according to the manufacturer's protocol.

Different combinations of degenerate primers were tried in the first and second PCR reactions on the DNA from each species until a combination of primers was found that resulted in specific amplification--meaning that a pronounced band of expected size (about 650-800 bp for NGH and GEG/GNG and 350-500 bp for NFP and PVM--sometimes accompanied by a few minor bands) was detected on agarose gel after two PCR reactions. The primer combinations of choice for different species of the Class Anthozoa are listed in Table 4. Some other primer combinations also resulted in amplification of fragments of correct size, but the sequence of these fragments showed no homology to the other fluorescent proteins identified or to *Aequorea victoria* GFP.

EXAMPLE 5**Obtaining Full-Length cDNA Copies**

Upon sequencing the obtained 3'-fragments of novel 5 fluorescent protein cDNAs, two nested 5'-directed primers were synthesized for cDNA (Table 5), and the 5' ends of the cDNAs were then amplified using two consecutive PCRs. In the next PCR reaction, the novel approach of "step-out PCR" was used to suppress background amplification. The step-out reaction mixture contained 1x Advantage 10 KlenTaq Polymerase Mix using buffer provided by the manufacturer (CLONTECH), 200 µM dNTPs, 0.2 µM of the first gene-specific primer (see Table 5), 0.02 µM of the T7-TS primer (SEQ ID No. 18), 0.1 µM of T7 primer (SEQ ID No. 19) and 1 µl of the 20-fold dilution of the amplified cDNA sample in a total volume of 20 µl. The cycling profile 15 was (Hybaid OmniGene Thermocycler, tube control mode): 23-27 cycles for 95°C, 10 sec.; 60°C, 30 sec.; 72°C, 40 sec. The product of amplification was diluted 50-fold in water and one µl of this dilution was added to the second (nested) PCR. The reaction contained 1X Advantage KlenTaq Polymerase Mix with provided buffer (CLONTECH), 20 200 µM dNTPs, 0.2 µM of the second gene-specific primer and 0.1 µM of TS primer (SEQ ID No. 2) in a total volume of 20 µl. The cycling profile was (Hybaid OmniGene Thermocycler, tube control mode): 12 cycles for 95°C, 10 sec.; 60°C, 30 sec.; 72°C, 40 sec. The product of amplification was then cloned into pAtlas vector (CLONTECH) according 25 to the manufacturer's protocol.

TABLE 5Gene-Specific Primers Used for 5'-RACE

Species	First Primer	Second (Nested) Primer
Anemonia majano	5'-GAAATAGTCAGGCATACTGGT (SEQ ID No. 20)	5'-GTCAGGCATAC TGGTAGGAT (SEQ ID No. 21)
Clavularia sp.	5'-CTTGAAATAGTCTGCTATATC (SEQ ID No. 22)	5'-TCTGCTATATC GTCTGGGT (SEQ ID No. 23)
Zoanthus sp.	5'- GTTCTTGAAATAGTCTACTATGT (SEQ ID No. 24)	5'-GTCTACTATGTCTT GAGGAT (SEQ ID No. 25)
Discosoma sp. "red"	5'-CAAGCAAATGGCAAAGGTC (SEQ ID No. 26)	5'-CGGTATTGTGGCC TTCGTA (SEQ ID No. 27)
Discosoma striata	5'-TTGTCTTCTTCTGCACAAC (SEQ ID No. 28)	5'-CTGCACAAACGG GTCCAT (SEQ ID No. 29)
Anemonia sulcata	5'-CCTCTATCTTCATTCCTGC (SEQ ID No. 30)	5'-TATCTTCATTCCT GCGTAC (SEQ ID No. 31)
Discosoma sp. "magenta"	5'-TTCAGCACCCCATCACGAG (SEQ ID No. 32)	5'-ACGCTCAGAGCTG GGTCC (SEQ ID No. 33)
Discosoma sp. "green"	5'-CCCTCAGCAATCCATCACGTT (SEQ ID No. 34)	5'-ATTATCTCAGTGGA TGGTTC (SEQ ID No. 35)

EXAMPLE 6Expression of nFPs in *E.coli*

5 To prepare a DNA construct for novel fluorescent protein expression, two primers were synthesized for each cDNA: a 5'-directed "downstream" primer with the annealing site located in the 3'-UTR of the cDNA and a 3'-directed "upstream" primer corresponding to the site of translation start site (not including the first ATG codon) (Table 10 6). Both primers had 5'-heels coding for a site for a restriction endonuclease; in addition, the upstream primer was designed so as to allow the cloning of the PCR product into the pQE30 vector (Qiagen) in such a way that resulted in the fusion of reading frames of the vector-encoded 6xHis-tag and nFP. The PCR was performed as follows: 1 µl of 15 the 20-fold dilution of the amplified cDNA sample was added to a mixture containing 1x Advantage KlenTaq Polymerase Mix with buffer provided by the manufacturer (CLONTECH), 200 µM dNTPs, 0.2 µM of upstream primer and 0.2 µM of downstream primer, in a final total volume of 20 µl. The cycling profile was (Hybaid OmniGene 20 Thermocycler, tube control mode): 23-27 cycles for 95°C, 10 sec.; 60°C, 30 sec.; 72°C, 40 sec. The product of this amplification step was purified by phenol-chlorophorm extraction and ethanol precipitation and then cloned into pQE30 vector using restriction endonucleases corresponding to the primers' sequence according to standard 25 protocols.

All plasmids were amplified in XL-1 blue *E. coli* and purified by plasmid DNA miniprep kits (CLONTECH). The recombinant clones were selected by colony color, and grown in 3 ml of LB medium (supplemented with 100 µg/ml of ampicillin) at 37°C overnight. 100 µl

of the overnight culture was transferred into 200 ml of fresh LB medium containing 100 µg/ml of ampicillin and grown at 37°C, 200 rpm up to OD₆₀₀ 0.6-0.7. 1 mM IPTG was then added to the culture and incubation was allowed to proceed at 37°C for another 16 hours. The 5 cells were harvested and recombinant protein, which incorporated 6x His tags on the N-terminus, was purified using TALON™ metal-affinity resin according to the manufacturer's protocol (CLONTECH).

TABLE 6

Primers Used to Obtain Full Coding Region of nFPs for Cloning into Expression Construct

Species	Upstream Primer	Downstream Primer
Anemonia majano	5' -acatggatccgctttcaaaca agtttatac (SEQ ID No. 36) BamHI	5'-tagtactcgagcttattcgta tttcagtgaaatc (SEQ ID No. 37) XhoI
Clavularia sp.	L: 5'-acatggatccaacatttttga gaaacg (SEQ ID No. 38) BamHI S: 5'-acatggatccaaagctctaacc accatg (SEQ ID No. 39) BamHI	5'-tagtactcgagcaacacaa accctcagacaaa (SEQ ID No. 40) XhoI
Zoanthus sp.	5'- acatggatccgctcagtcagg cacgg (SEQ ID No. 41) BamHI	5'-tagtactcgagggttggaaactacat tcttatca (SEQ ID No. 42) XhoI
Discosoma sp. "red"	5'- acatggatccaggcgttccaagaat gttatac (SEQ ID No. 43) BamHI	5'-tagtactcgaggaggccaagttc agccta (SEQ ID No. 44) XhoI
Discosoma striata	5'- acatggatccaggcgttccaaggatgtg (SEQ ID No. 45) BamHI	5'-tagcgagctcttatcatgcctc gtcacct (SEQ ID No. 46) SacI
Anemonia sulcata	5'- acatggatccgctccctttaaagaagact (SEQ ID No. 47) BamHI	5'-tagtactcgagtcctgggagc ggcttg (SEQ ID No. 48) XhoI
Discosoma sp. "magenta"	5'- acatggatccaggcgttccaagaatgtgat (SEQ ID No. 49) BamHI	5'-tagtactcgaggccattacg ctaatac (SEQ ID No. 50) XhoI
Discosoma sp. "green"	5'-acatggatccaggcgtgcacttaagaagaaatg (SEQ ID No. 51)	5'-tagtactcgagattcggttaat gccttg (SEQ ID No. 52)

EXAMPLE 7**Novel Fluorescent Proteins and cDNAs Encoding the Proteins**

Seven cDNA full-length cDNAs encoding fluorescent proteins were obtained (SEQ ID Nos. 45-51), and seven novel fluorescent proteins were produced (SEQ ID Nos. 53-59). The spectral properties of the isolated novel fluorescent proteins are shown in Table 7, and the emission and excitation spectra for the novel proteins are shown in Figures 3-11.

TABLE 7Spectral Properties of the Isolated NFPs.

Species	NFP Name	Abs. Max. n m	Emission Maximum n m	Maximum Extinction Coeff.	Relative Quantum Yield*	Relative Brightness **
Anemonia majano	amFP486	458	486	40,000	0.3	0.43
Clavularia sp.	cFP484	456	484	35,300	0.6	0.77
Zoanthus sp.	zFP506	496	506	35,600	0.79	1.02
Zoanthus sp.	zFP538	528	538	20,200	0.52	0.38
Discosoma sp. "red"	drFP583	558	583	22,500	0.29	0.24
Discosoma striata	dsFP483	443	483	23,900	0.57	0.50
Anemonia sulcata	asFP600	572	596	56,200	<0.001	-
Discosoma sp "green"	dgFP512	502	512	20,360	0.3	0.21
Discosoma sp. "magenta"	dmFP592	573	593	21,800	0.11	0.09

5 *relative quantum yield was determined as compared to the quantum yield of *A. victoria* GFP.

**relative brightness is extinction coefficient multiplied by quantum yield divided by the same value for *A. victoria* GFP.

Multiple alignment of fluorescent proteins is shown in Figure 2A. The numbering is based on *Aequorea victoria* green fluorescent protein (GFP, SEQ ID No. 54). The amino acid sequences of the novel fluorescent proteins are labeled as SEQ ID Nos. 55-63. Two 5 proteins from *Zoanthus* and four from *Discosoma* are compared between each other: residues identical to the corresponding ones in the first protein of the series are represented by dashes. Introduced gaps are represented by dots. In the sequence of *A. victoria* GFP, the stretches forming β -sheets are underlined; the residues whose side 10 chains form the interior of the β -can are shaded. Figure 2B shows the N-terminal part of cFP484, which has no homology with the other proteins. The putative signal peptide is underlined.

The following references were cited herein.

1. Ormo et al., (1996) Science 273: 1392-1395.
- 15 2. Yang, F., et al., (1996) Nature Biotech 14: 1246-1251.
3. Cormack, et al., (1996) Gene 173, 33-38.
4. Haas, et al., (1996) Current Biology 6, 315-324.
5. Yang, et al., (1996) Nucleic Acids Research 24, 4592-4593.
6. Ghoda, et al.. (1990) J. Biol. Chem. 265: 11823-11826.
- 20 7. Prasher D.C. et al. (1992) Gene 111:229-33.
8. Kain et al. (1995) Biotechniques 19(4):650-55.
9. Chomczynski P., et al., (1987) Anal. Biochem. 162, 156-159.
10. Frohman et al., (1998) PNAS USA, 85, 8998-9002.

Any patents or publications mentioned in this specification 25 are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, 5 molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope 10 of the claims.

WHAT IS CLAIMED IS:

1. A method of identifying a DNA sequence encoding a fluorescent protein, comprising the step of:

5 screening for an existence of a nucleic acid sequence in a sample, wherein said nucleic acid sequence encodes a peptide having a sequence selected from the group consisting of SEQ ID Nos. 3, 5, 8, 11, 12 and 14, and wherein the existence of said nucleic acid sequence identifies the DNA sequence encoding the fluorescent protein.

10

2. A method of identifying a DNA sequence encoding a fluorescent protein, comprising the step of:

screening for an existence of a nucleic acid sequence in a sample, wherein said nucleic acid sequence hybridizes to a primer selected from the group consisting of SEQ ID Nos. 4, 6, 7, 9, 10, 13, 15 and 16, and wherein the existence of said nucleic acid sequence identifies the DNA sequence encoding the fluorescent protein.

15

3. A method of analyzing a fluorescent protein in a cell, comprising the steps of:

a) expressing a nucleic acid sequence encoding a fluorescent protein in said cell, wherein said protein having an amino acid sequence selected from the group consisting of SEQ ID Nos. 55-63; and

25

b) measuring a fluorescence signal from said protein.

4. The method of claim 3, further comprising the step of:

sorting said cell according to said signal.

5. The method of claim 4, wherein said step of sorting comprises sorting said cell by fluorescence activated cell sorting.

5 6. The method of claim 3, wherein said nucleic acid sequence comprises a gene of interest encoding a protein of interest fused to said fluorescent protein, wherein said protein of interest is distinct from said fluorescent protein.

10 7. The method of claim 6, wherein the fluorescence signal indicates a presence of said gene of interest in said cell.

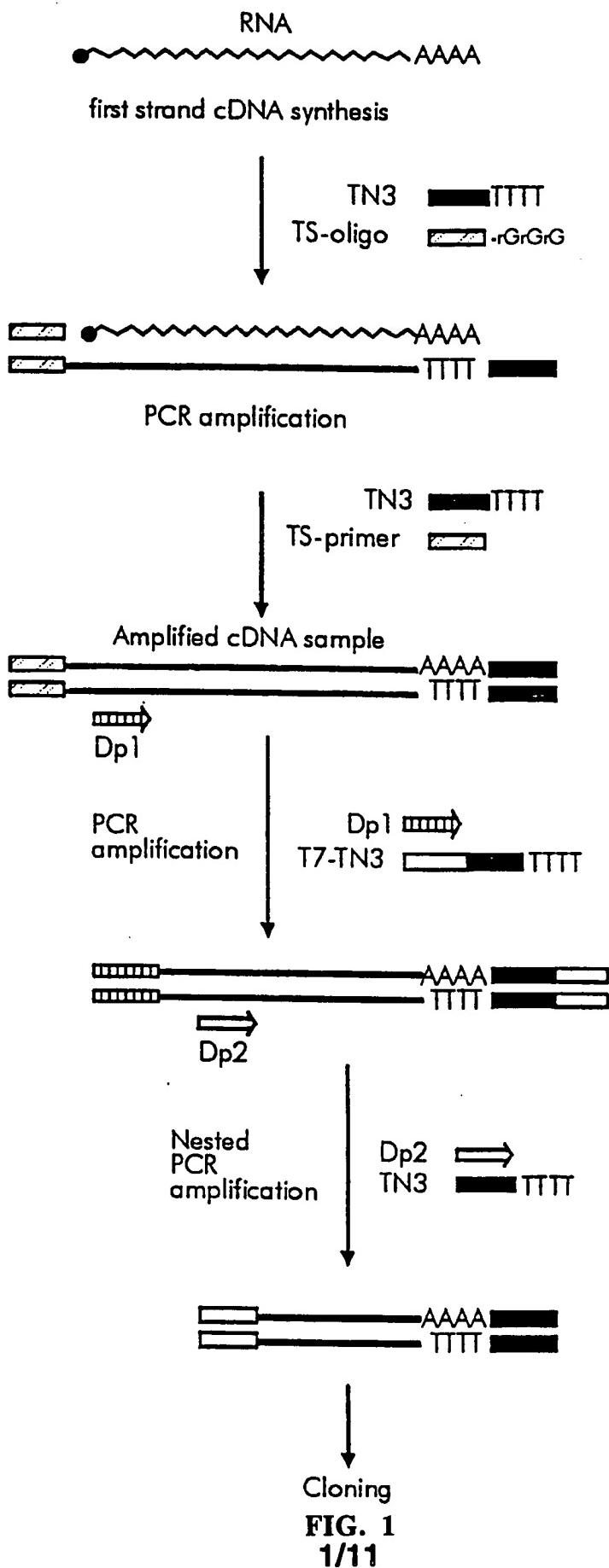
8. The method of claim 7, wherein said cell further comprises a protein of interest fused to said fluorescent protein.

15

9. The method of claim 8, further comprising the step of:

identifying an intracellular location of said fluorescent protein, thereby identifying an intracellular location of said protein of
20 interest.

10. An isolated and purified fluorescent protein selected from the group consisting of amFP486, cFP484, zFP506, zFP538, dsFP483, drFP583, asFP600, dgFP512 and dmFP592.



10	20	30	40	50		SEQ ID#
<u>MSKGEELFTG. VVPILVELGDVNGHKFSVSGEGEGLDATYGKLTLKFICTT. GKLPVP..W GFP</u>						54
MAQSKHGLTK. EMTMKYRMEGCVGDHKFVITGEGIGYPFKGKQAINLCVV..EGGPLPFAE zFP506						57
--H----KE.-----H----N-----T----I.-----S- zFP538						58
MSWSKSVIKE. EMLIDLHLEGTFNIGHYFEIKGKGKGKPNEGNTVTLEV..KGGPLPFGW dsFP483						59
....M-AL--.Y-K-N-TM--VV--LP-K-R-D---YQ-SQEL--T-V.-----SY dgFP512						62
-RS--N---.F-RFKVRM--V--E--E-E-R-Y--H--K-K--.-----A- drFP583						60
M-C--N---.F-RFKVRM--V--E--E-E-R-Y--HCS-K-M--.-----AF dmFP592						63
...MASFLKK. TMPFKTTIEGTVNIGHYFKCTGKGEGNPFEGTQEMKIEVI..EGGPLPFAF asFP600						61
MALSNKFIGD. DMKMTYIIMDGVNIGHYFTVKGEENGKPYEGTQTSTFKVTMANGGPLAFSF amFP486						55
«KALTTMGVIKPDMMIKLKMEGNVNHAHVIEGEGEKGPKYDGHTLNLEVVKMAEGAPLPFSY cFP484						56
60	70	80	90	100	110	
<u>PTLVTTFSYGVQCFSRYPDHMKQHDFFKSAM..:PEGYVQERTIFFKDDGNYKTRAEVKFEGD..</u>						GFP
DILSAAFNYGNRVEYTEYPQDIV..DYFKNSC...PAGYTWDRSFLFEDGAVCICNADITVSVEEN						zFP506
----G-K--D-I-----G-----V-----K--						zFP538
HILCPQFQYGNKAFVHHPPDDIP..DYLKLSF...PEGYTWEERSMHFEDGGLCCITNDISLTGN..						dsFP483
D--TTM----R--NY-E--..IF-QTCSPGNG--S-Q-T-TY---V-TA-SN--VV-D..						dgFP512
D--S----S-VY-K--A--..K----FK--V-N----VVTV-Q-S--QDG..						drFP583
D--S----S-VY-K--A--..K----FK--V-N----VVTVSQ-S--,KD..						dmFP592
HILSTSCMYGSKTFIKYVSGIP..DYFKQSF...PEGFTWERTTYEDGGFLTAHQDTSLDG..						asFP600
DILSTVFKYGNRCFTAYPTSM..DYFKQAF..PDGMSYERTFTYEDGGVATASWEISLKG..						amFP486
DILSNQYGNRALTKYPDDIA..DYFKQSF...PEGYSWERTMTFEDKGIVKVKSDISMEED..						cFP484
120	130	140	150	160	170	
<u>TLVNRIELKGIDFKEDGNILGHKLEYNNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQL</u>						GFP
CMYHESKFYGVNFPADGPVM. KKMTDNWEPSCEKIIIPVKQGILKGDVSMYLLKDGGR..R						zFP506
-I--K-I-N-M-----T--A--M-----Y-						zFP538
CQNYDIKFTGLNFPPNGPVV.QKKTTGWEPSTERLYP..RDGVLIQDIHHALTVEGGGHYV						dsFP483
T----H-M-A--LD--MM--R-MK----IMFE ---L-R-D-AMS-LLK----R						dgFP512
--I-KV--I-V--SD--M.----M--A-----K-E--K--KLKD---L						drFP583
--I-EV--I-V--SD--M.-RR-R----S-----K--M--RL----L						dmFP592
CLVYKVKILGNNFPADEGPVM.QNKAGRWEPAIEIVYE..VDGVLRGQSLSMALKCPGGRHLT						asFP600
CFEHKSTFHGVNFPADGPVM.AKKTGWDPSFEKMTV:.CDGILKGDVTAFLMLQGGGNR						amFP486
SFIFYEIRFDGMNFPPNGPVM.QKTLKWEPESTEIMYV,.RDGVLVGDISHSSLLEGGGHYR						cFP484
180	190	200	210	220	230	
<u>ADHYQONTPIGDG.PVLLPDNHYLSTOSALSKDPNEKRDHMVLLFVTAAGITHGMDELYK</u>						GFP
CQFDVTYKAKSV..PRKMPDWHFIQHKLTREDRSDAKNQKWLTEHAIASGSALP						zFP506
-----S--E-----L-----Q-----FP---A						zFP538
CDIKTVYPAKK...PVKMPGYHYVDTKLVIR SNDKEFM.KVEEHEIAVARHHPLQSQ						dsFP483
--FE-I-KPN- V----D--F--HYIE-T-QQNYN V--LT-V-E--YSS-EKIGKSKA						dgFP512
VEF-SI-M----QL--Y--S--D-T-HNEDYT.I--QY-RTEG---LFL						drFP583
VEF-SI-MV-- PS-QL--Y--S--DMT-HNEDYT V--QY-KTQ----FIKPLQ						dmFP592
CHLHTTYRSKKPASALKMPGFHEDHRIEIMEEVEKGK.CYKQYEAAVGRYCDAAPSKLGHN						asFP600
CQFHTSYKTKK...PVTMPPNHVVEHRIARTDLDKGGN.SVQLTEHAVAHITSVFPF						amFP486
CDFKSIYKAKK...VVKLVDYHFVDRRIEILNHDKDYN.KVTLYENAVARYSLPSQA						cFP484

FIG. 2A

»

MKCKFVFCLSFLVLAITNANI FLRNEADLEEKTLRIP

FIG. 2B

2/11

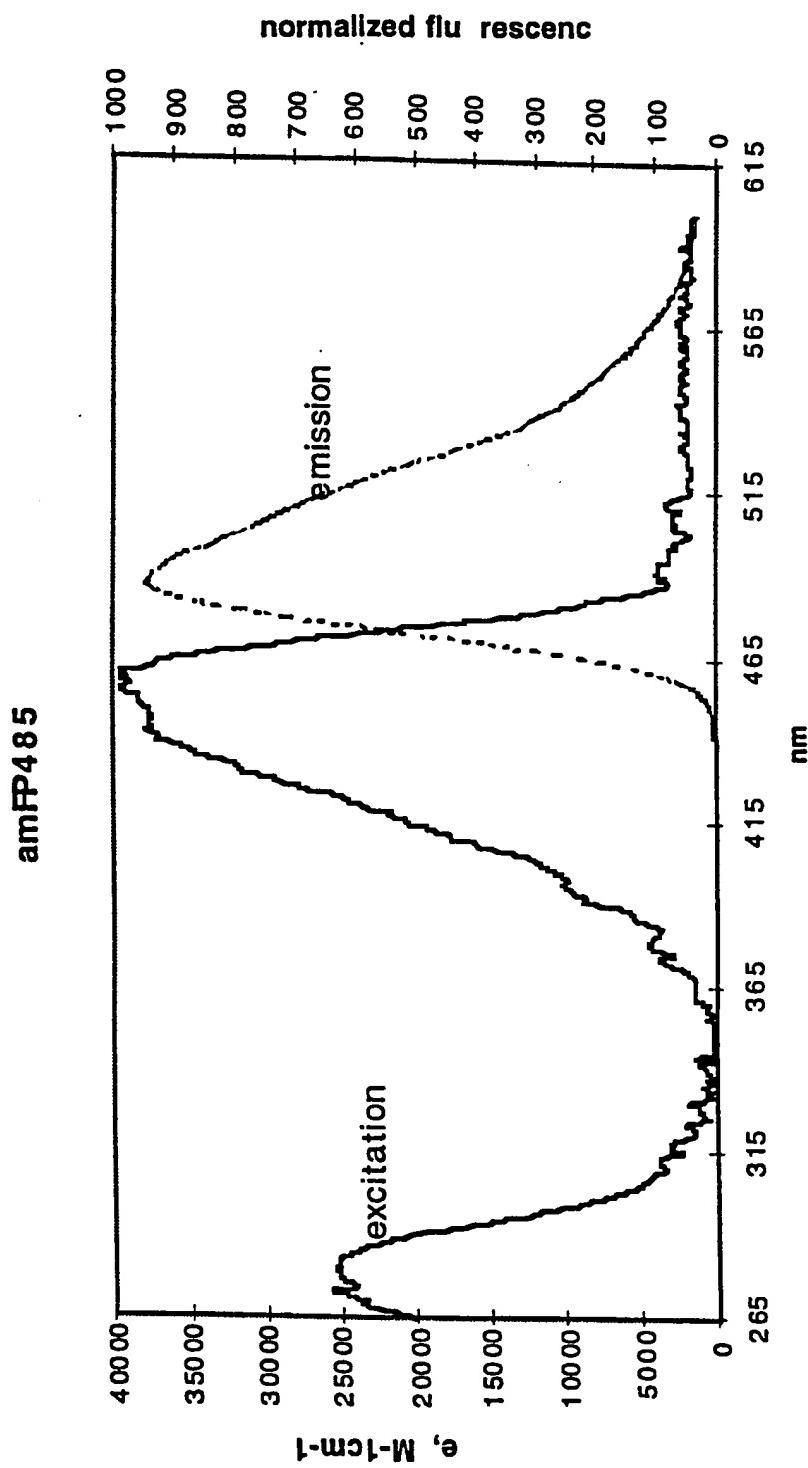


FIG. 3

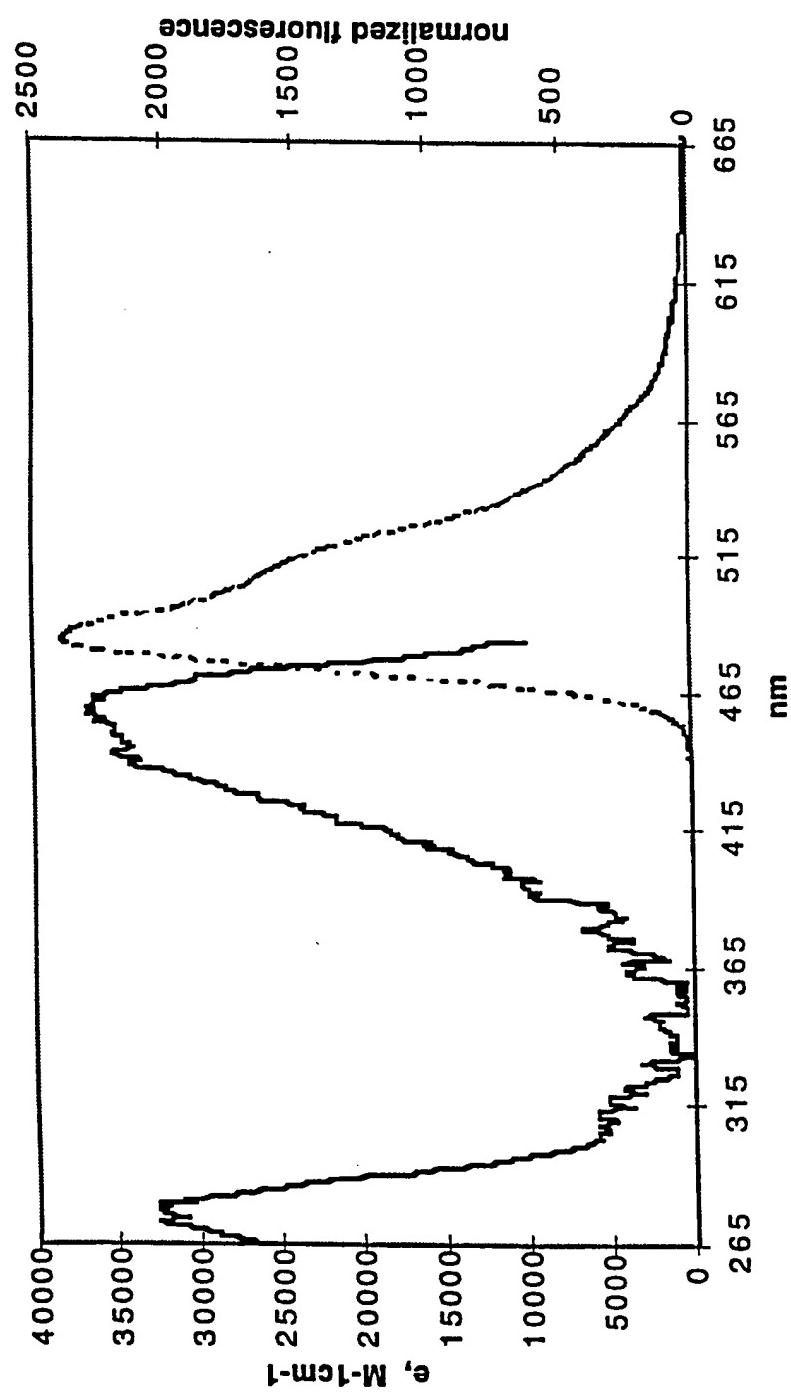


FIG. 4

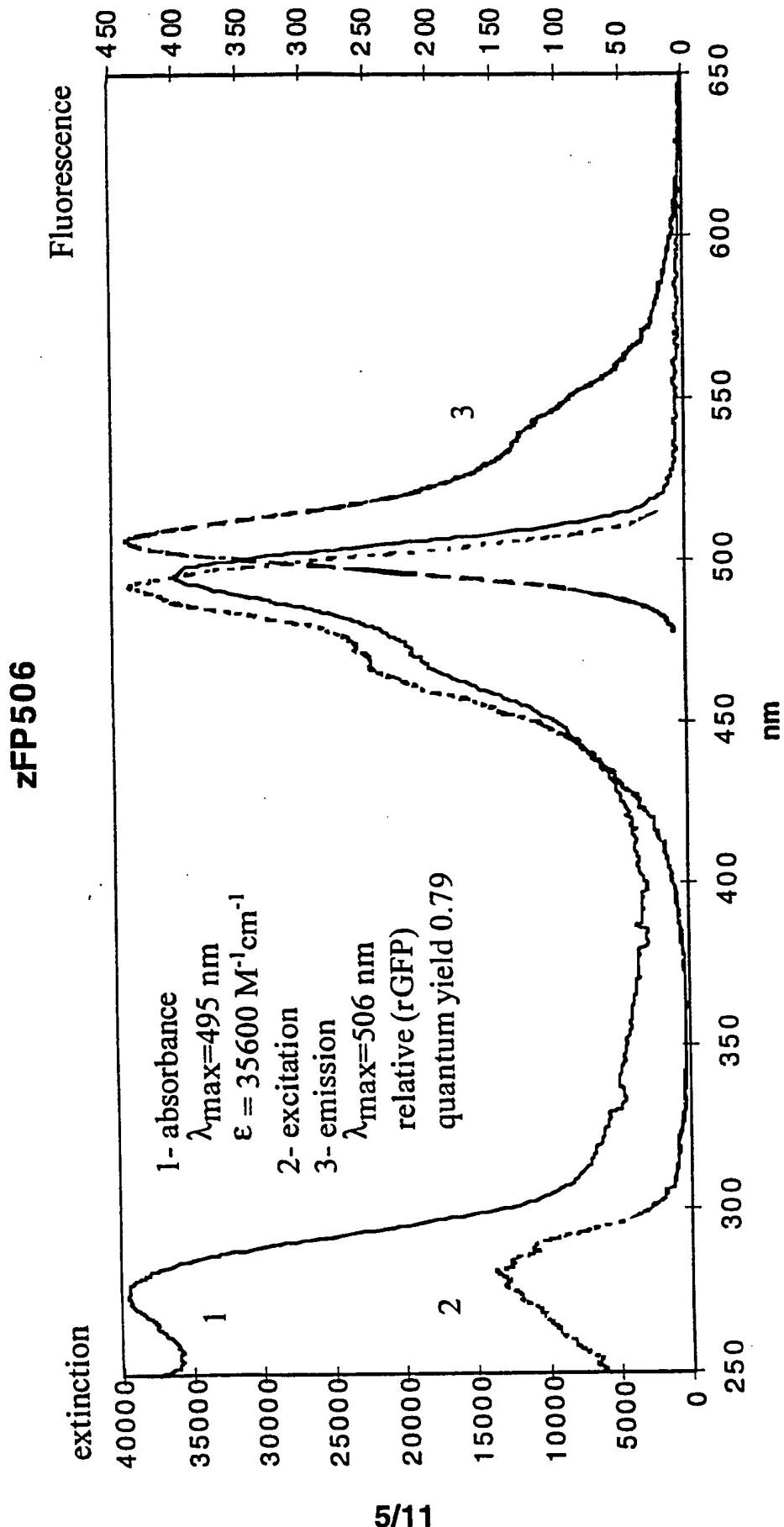
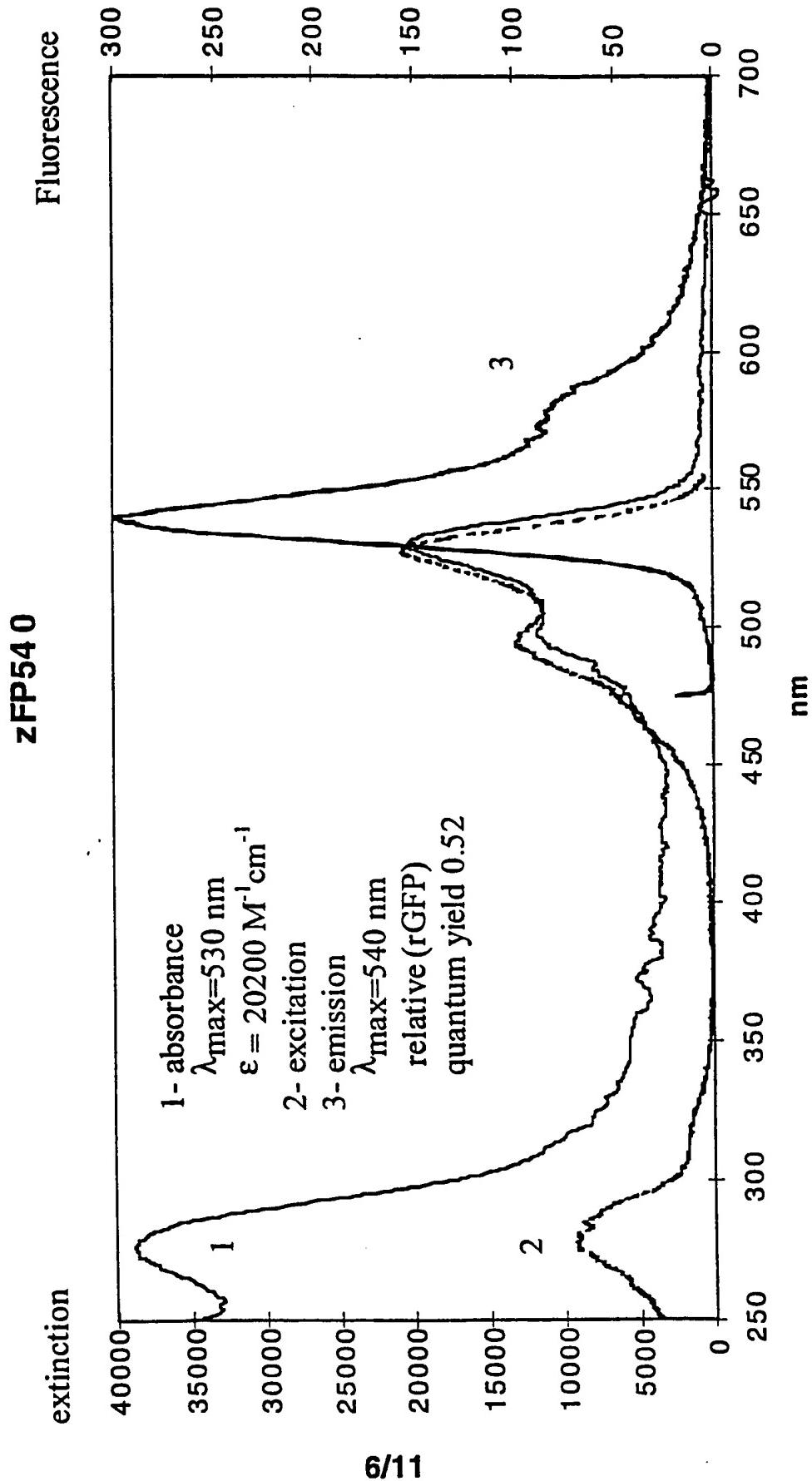
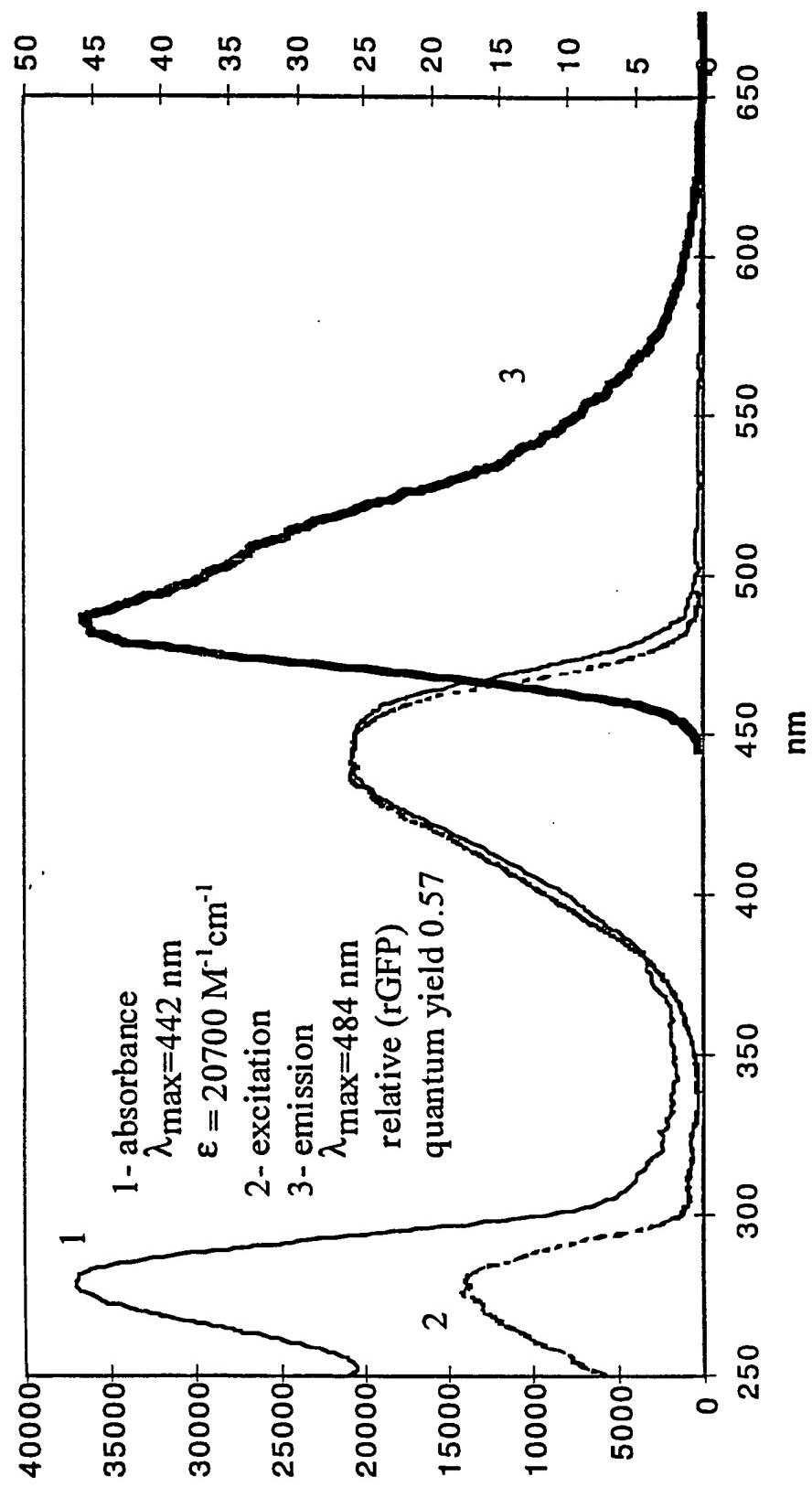


FIG. 5

**FIG. 6**

dsFP484**FIG. 7**

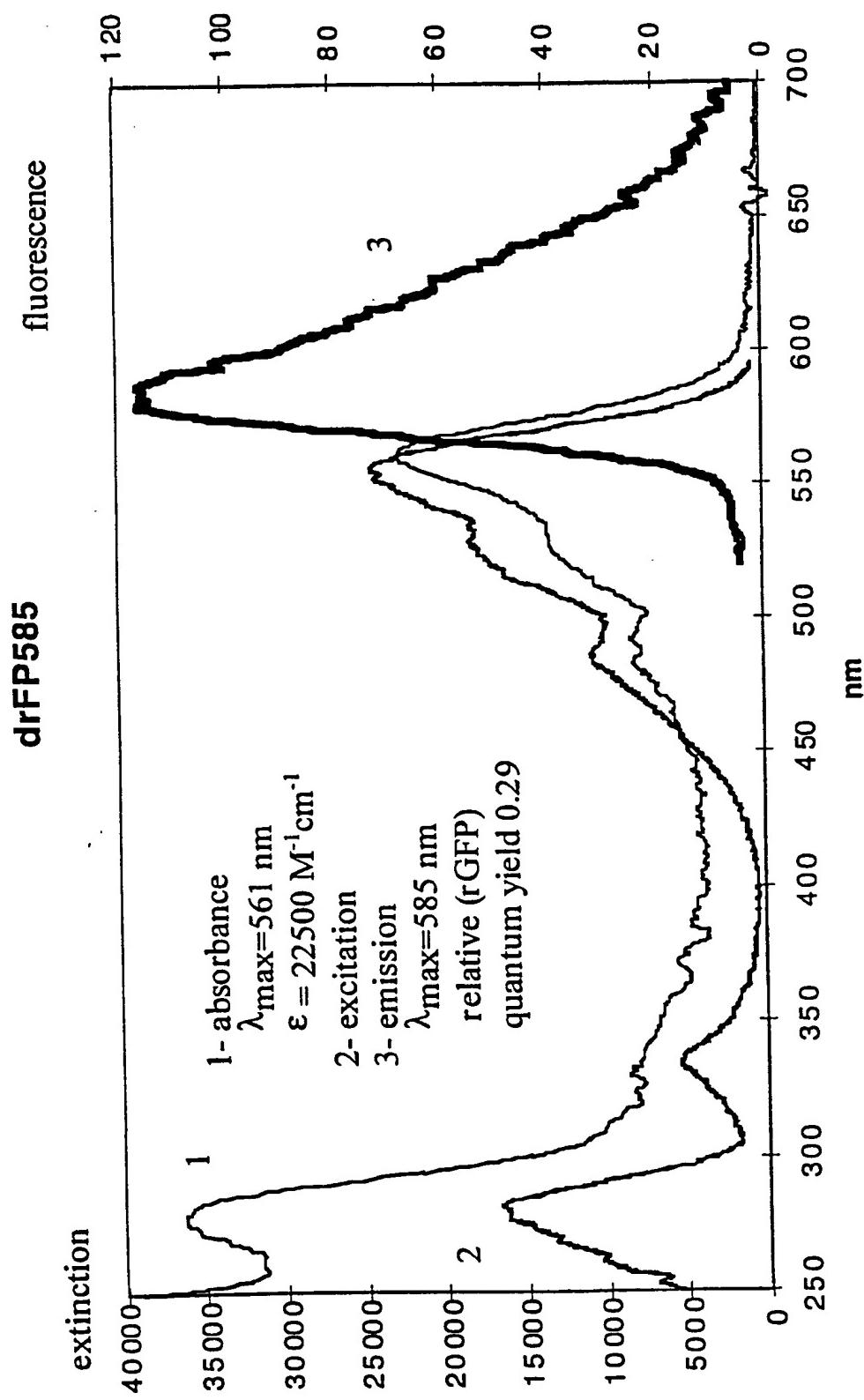


FIG. 8

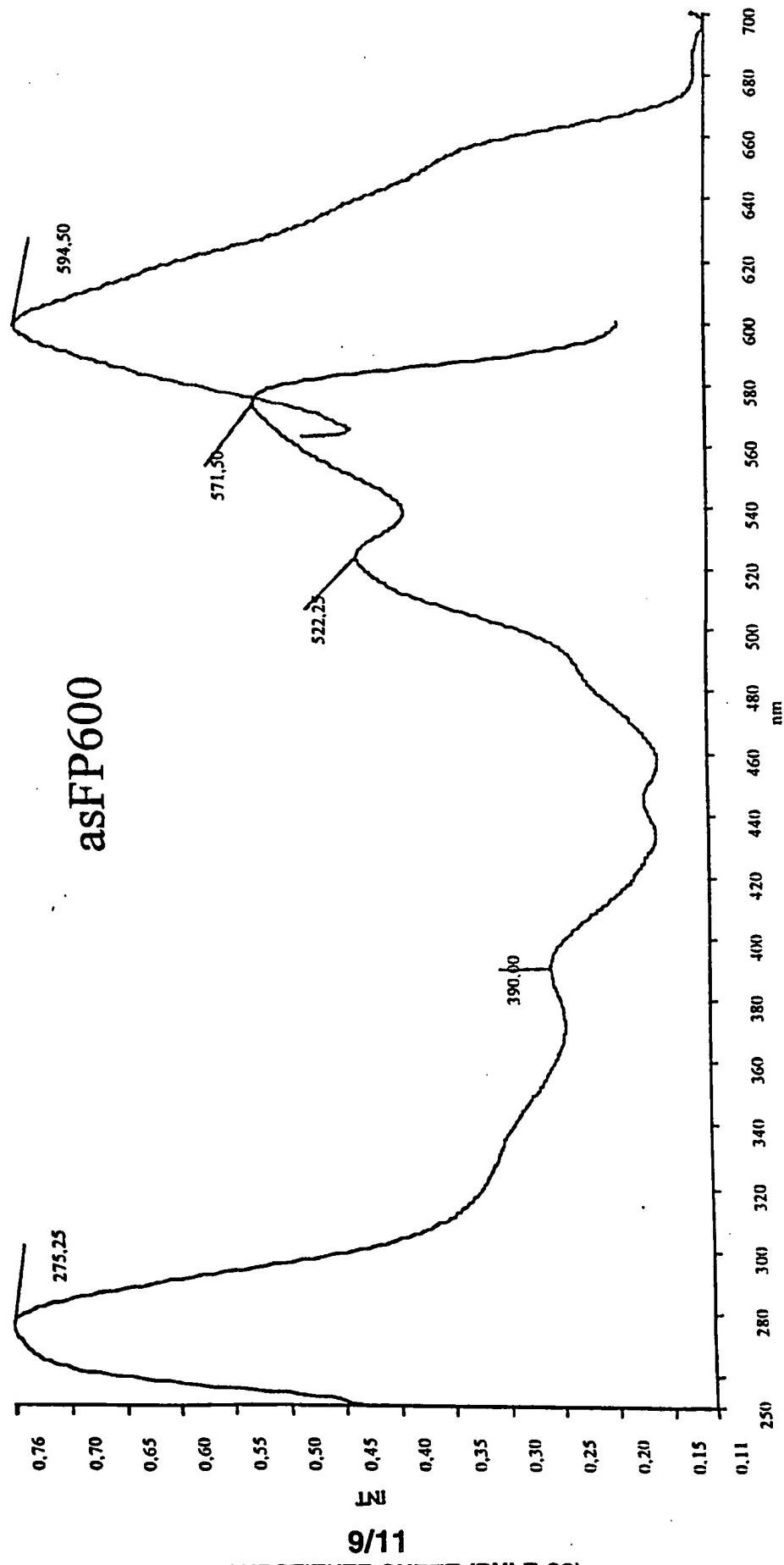


FIG. 9

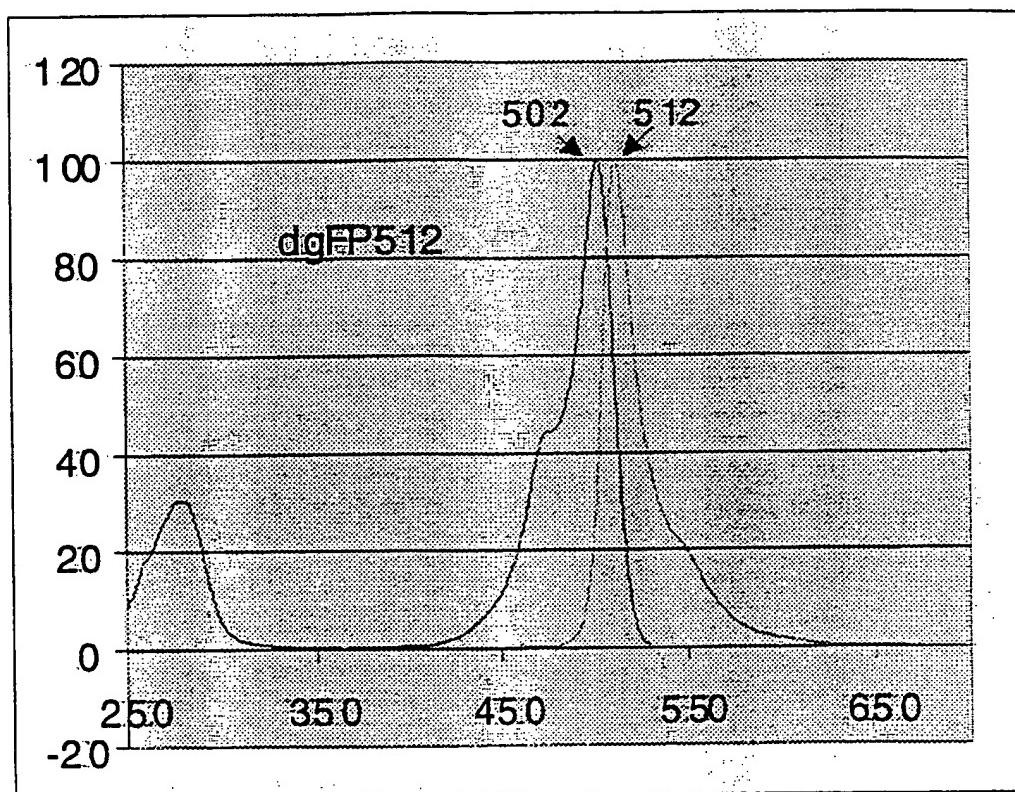


Fig. 10

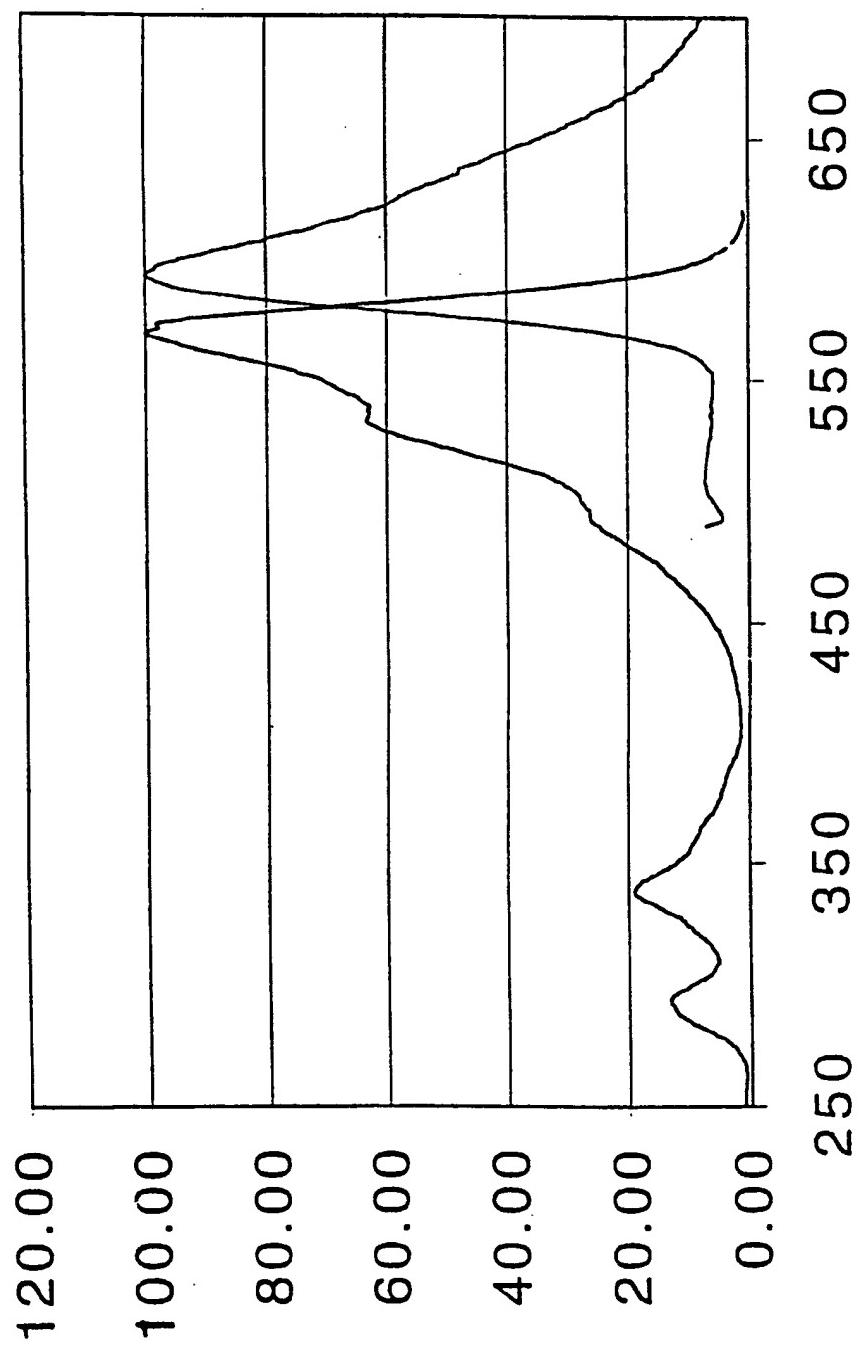


FIG. 11

SEQUENCE LISTING

<110> Lukyanov, Sergey A.
Labas, Yulii A.
Matz, Mikhail V.
5 Fradkov, Arcady F.

<120> Fluorescent proteins from non-bioluminescent
species of Class Anthozoa, genes encoding such
proteins and uses thereof

<130> D6196PCT

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5

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20

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5

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protein
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5

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5

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	<211>	21	
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	<222>	15	
	<223>	primer PVM _b used for isolation of fluorescent protein; n at position 15 represents any of the four bases	
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	<220>		
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5 <213> artificial sequence
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30 *Anemonia majano*
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19

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		<i>Discosoma striata</i>
	<400>	28
	ttgtcttctt ctgcacaac	
		19
5	<210>	29
	<211>	17
	<212>	DNA
	<213>	artificial sequence
	<220>	
10	<221>	primer_bind
	<223>	gene-specific primer used for 5'-RACE for
	<i>Discosoma striata</i>	
	<400>	29
	ctgcacaacg ggtccat	
		17
15		
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	<211>	20
	<212>	DNA
	<213>	artificial sequence
20	<220>	
	<221>	primer_bind
	<223>	gene-specific primer used for 5'-RACE for
	<i>Anemonia sulcata</i>	
	<400>	30
25	cctcttatctt catttcctgc	
		20
	<210>	31
	<211>	20
	<212>	DNA
30	<213>	artificial sequence
	<220>	
	<221>	primer_bind
	<223>	gene-specific primer used for 5'-RACE for
	<i>Anemonia sulcata</i>	
35	<400>	31
	tatcttcatt tcctgcgtac	
		20

<210> 32
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<212> DNA
5 <213> artificial sequence
<220>
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Discosoma sp. "magenta"
10 <400> 32

ttcagcaccc catcacgag 19

<210> 33
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15 <212> DNA
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20 <223> *Discosoma sp.* "magenta"
<400> 33

acgctcagag ctgggttcc 19

<210> 34
25 <211> 22
<212> DNA
<213> artificial sequence
<220>
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30 <223> gene-specific primer used for 5'-RACE for
Discosoma sp. "green"
<400> 34

ccctcagcaa tccatcacgt tc 22

35 <210> 35
<211> 20
<212> DNA

<213> artificial sequence
<220>
<221> primer_bind
<223> gene-specific primer used for 5'-RACE for
5 Discosoma sp. "green"
<400> 35

attatctcag tggatggttc 20

<210> 36
10 <211> 31
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<213> artificial sequence
<220>
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15 <223> upstream primer used to obtain full coding region
of nFPs from Anemonia majano
<400> 36

acatggatcc gctcttcaa acaagtttat c 31

20 <210> 37
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<213> artificial sequence
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25 <221> primer_bind
<223> downstream primer used to obtain full coding
region of nFPs from Anemonia majano
<400> 37

tagtactcga gcttattcgt atttcagtga aatc 34
30

<210> 38
<211> 29
<212> DNA
<213> artificial sequence
35 <220>
<221> primer_bind
<223> upstream primer used to obtain full coding region

		of nFPs from <i>Clavularia sp.</i>
<400>	38	
		acatggatcc aacattttt tgagaaacg
		29
5	<210>	39
	<211>	28
	<212>	DNA
	<213>	artificial sequence
	<220>	
10	<221>	primer_bind
	<223>	upstream primer used to obtain full coding region of nFPs from <i>Clavularia sp.</i>
	<400>	39
		acatggatcc aaagctctaa ccaccatg
		28
15	<210>	40
	<211>	31
	<212>	DNA
	<213>	artificial sequence
20	<220>	
	<221>	primer_bind
	<223>	downstream primer used to obtain full coding region of nFPs from <i>Clavularia sp.</i>
	<400>	40
25	tagtactcga gcaacacaaa ccctcagaca a	31
	<210>	41
	<211>	28
	<212>	DNA
30	<213>	artificial sequence
	<220>	
	<221>	primer_bind
	<223>	upstream primer used to obtain full coding region of nFPs from <i>Zoanthus sp.</i>
35	<400>	41
		acatggatcc gtcgtgtcaa agcacggt
		28

<210> 42
<211> 32
<212> DNA
5 <213> artificial sequence
<220>
<221> primer_bind
<223> downstream primer used to obtain full coding
region of nFPs from *Zoanthus sp.*
10 <400> 42

tagtactcga ggttggaact acattcttat ca 32

<210> 43
<211> 31
15 <212> DNA
<213> artificial sequence
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<221> primer_bind
<223> upstream primer used to obtain full coding region
20 of nFPs from *Discosoma sp.* "red"
<400> 43

acatggatcc aggtcttcca agaatgttat c 31

<210> 44
25 <211> 29
<212> DNA
<213> artificial sequence
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<221> primer_bind
30 <223> downstream primer used to obtain full coding
region of nFPs from *Discosoma sp.* "red"
<400> 44

tagtactcga ggagccaagt tcagcctta 29

35 <210> 45
<211> 28
<212> DNA

5 <213> artificial sequence
<220>
<221> primer_bind
<223> upstream primer used to obtain full coding region
of nFPs from *Discosoma striata*
<400> 45

acatggatcc agttggtcca agagtgtg 28

10 <210> 46
<211> 28
<212> DNA
<213> artificial sequence
<220>
<221> primer_bind
<223> downstream primer used to obtain full coding
region of nFPs from *Discosoma striata*
<400> 46

15 tagcgagctc tatcatgcct cgtcacct 28

20 <210> 47
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of nFPs from *Anemonia sulcata*
<400> 47

25 acatggatcc gcttccttt taaagaagac t 31

30 <210> 48
<211> 28
<212> DNA
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35

		region of nFPs from <i>Anemonia sulcata</i>
	<400>	48
	tagtactcgatgccttggacgcggcttg	28
5	<210>	49
	<211>	30
	<212>	DNA
	<213>	artificial sequence
	<220>	
10	<221>	primer_bind
	<223>	upstream primer used to obtain full coding region of nFPs from <i>Discosoma sp.</i> "magenta"
	<400>	49
	acatggatcc agttgttcca agaatgtgat	30
15		
	<210>	50
	<211>	26
	<212>	DNA
	<213>	artificial sequence
20	<220>	
	<221>	primer_bind
	<223>	downstream primer used to obtain full coding region of nFPs from <i>Discosoma sp.</i> "magenta"
	<400>	50
25	tagtactcgatggcattacg ctaatc	26
	<210>	51
	<211>	31
	<212>	DNA
30	<213>	artificial sequence
	<220>	
	<221>	primer_bind
	<223>	upstream primer used to obtain full coding region of nFPs from <i>Discosoma sp.</i> "green"
35	<400>	51
	acatggatcc agtgcactta aagaagaaat g	31

	<210>	52	
	<211>	29	
	<212>	DNA	
5	<213>	artificial sequence	
	<220>		
	<221>	primer_bind	
	<223>	downstream primer used to obtain full coding region of nFPs from <i>Discosoma sp.</i> "green"	
10	<400>	52	
	tagtactcga gattcggttt aatgccttg		29
	<210>	53	
	<211>	33	
15	<212>	DNA	
	<213>	artificial sequence	
	<220>		
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	<223>	TS-oligo used in cDNA synthesis and RACE	
20	<400>	53	
	aagcagtggt atcaacgcag agtacgcrgr grg		33
	<210>	54	
	<211>	238	
25	<212>	PRT	
	<213>	<i>Aequorea victoria</i>	
	<220>		
	<223>	amino acid sequence of GFP	
	<400>	54	
30	Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu		
	5	10	15
	Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser		
	20	25	30
	Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys		
35	35	40	45
	Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu		
	50	55	60

Val	Thr	Thr	Phe	Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	
			65			70						75			
Asp	His	Met	Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	
			80				85					90			
5	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn
			95					100				105			
Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	
			110					115				120			
Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	
10			125					130				135			
Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	
			140					145				150			
Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	
			155					160				165			
15	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp
			170					175				180			
His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	
			185					190				195			
Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	
20			200					205				210			
Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	
			215					220				225			
Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys			
			230					235							
25															
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	<211>		229												
	<212>		PRT												
	<213>		Anemonia majano												
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	<223>		amino acid sequence of amFP486												
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							5			10			15		
35	Tyr	His	Met	Asp	Gly	Cys	Val	Asn	Gly	His	Tyr	Phe	Thr	Val	Lys
							20			25			30		
	Gly	Glu	Gly	Asn	Gly	Lys	Pro	Tyr	Glu	Gly	Thr	Gln	Thr	Ser	Thr
							35			40			45		

	Phe Lys Val Thr Met Ala Asn Gly Gly Pro Leu Ala Phe Ser Phe		
	50	55	60
	Asp Ile Leu Ser Thr Val Phe Lys Tyr Gly Asn Arg Cys Phe Thr		
	65	70	75
5	Ala Tyr Pro Thr Ser Met Pro Asp Tyr Phe Lys Gln Ala Phe Pro		
	80	85	90
	Asp Gly Met Ser Tyr Glu Arg Thr Phe Thr Tyr Glu Asp Gly Gly		
	95	100	105
	Val Ala Thr Ala Ser Trp Glu Ile Ser Leu Lys Gly Asn Cys Phe		
10	110	115	120
	Glu His Lys Ser Thr Phe His Gly Val Asn Phe Pro Ala Asp Gly		
	125	130	135
	Pro Val Met Ala Lys Lys Thr Thr Gly Trp Asp Pro Ser Phe Glu		
	140	145	150
15	Lys Met Thr Val Cys Asp Gly Ile Leu Lys Gly Asp Val Thr Ala		
	155	160	165
	Phe Leu Met Leu Gln Gly Gly Asn Tyr Arg Cys Gln Phe His		
	170	175	180
	Thr Ser Tyr Lys Thr Lys Lys Pro Val Thr Met Pro Pro Asn His		
20	185	190	195
	Val Val Glu His Arg Ile Ala Arg Thr Asp Leu Asp Lys Gly Gly		
	200	205	210
	Asn Ser Val Gln Leu Thr Glu His Ala Val Ala His Ile Thr Ser		
	215	220	225
25	Val Val Pro Phe		

	<210>	56	
	<211>	266	
30	<212>	PRT	
	<213>	Clavularia sp.	
	<220>		
	<223>	amino acid sequence of cFP484	
	<400>	56	
35	Met Lys Cys Lys Phe Val Phe Cys Leu Ser Phe Leu Val Leu Ala		
	5	10	15
	Ile Thr Asn Ala Asn Ile Phe Leu Arg Asn Glu Ala Asp Phe Glu		

	20	25	30
	Glu Lys Thr Phe Arg Ile Pro Lys Ala Leu Thr Thr Met Gly Val		
	35	40	45
	Ile Lys Pro Asp Met Lys Ile Lys Leu Lys Met Glu Gly Asn Val		
5	50	55	60
	Asn Gly His Ala Phe Val Ile Glu Gly Glu Gly Glu Lys Pro		
	65	70	75
	Tyr Asp Gly Thr His Thr Leu Asn Leu Glu Val Lys Glu Gly Ala		
	80	85	90
10	Pro Leu Pro Phe Ser Tyr Asp Ile Leu Ser Asn Ala Phe Gln Tyr		
	95	100	105
	Gly Asn Arg Ala Leu Thr Lys Tyr Pro Asp Asp Ile Ala Asp Tyr		
	110	115	120
	Phe Lys Gln Ser Phe Pro Glu Gly Tyr Ser Trp Glu Arg Thr Met		
15	125	130	135
	Thr Phe Glu Asp Lys Gly Ile Val Lys Val Lys Ser Asp Ile Ser		
	140	145	150
	Met Glu Glu Asp Ser Phe Ile Tyr Glu Ile Arg Phe Asp Gly Met		
	155	160	165
20	Asp Phe Pro Pro Asn Gly Pro Val Met Gln Lys Lys Thr Leu Lys		
	170	175	180
	Trp Glu Pro Ser Thr Glu Ile Met Tyr Val Arg Asp Gly Val Leu		
	185	190	195
	Val Gly Asp Ile Ser His Ser Leu Leu Leu Glu Gly Gly His		
25	200	205	210
	Tyr Arg Cys Asp Phe Lys Ser Ile Tyr Lys Ala Lys Lys Val Val		
	215	220	225
	Lys Leu Pro Asp Tyr His Phe Val Asp His Arg Ile Glu Ile Leu		
	230	235	240
30	Asn His Asp Lys Asp Tyr Asn Lys Val Thr Leu Tyr Glu Asn Ala		
	245	250	255
	Val Ala Arg Tyr Ser Leu Leu Pro Ser Gln Ala		
	260	265	
35	<210>	57	
	<211>	230	
	<212>	PRT	

<213> Zoanthus sp.
 <220>
 <223> amino acid sequence of zFP506
 <400> 57

5	Ala Gln Ser Lys His Gly Leu Thr Lys Glu Met Thr Met Lys Tyr		
	5	10	15
	Arg Met Glu Gly Cys Val Asp Gly His Lys Phe Val Ile Thr Gly		
	20	25	30
	Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Ala Ile Asn Leu		
10	35	40	45
	Cys Val Val Glu Gly Gly Pro Leu Pro Phe Ala Glu Asp Ile Leu		
	50	55	60
	Ser Ala Ala Phe Asn Tyr Gly Asn Arg Val Phe Thr Glu Tyr Pro		
	65	70	75
15	Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly Tyr		
	80	85	90
	Thr Trp Asp Arg Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile		
	95	100	105
	Cys Asn Ala Asp Ile Thr Val Ser Val Glu Glu Asn Cys Met Tyr		
20	110	115	120
	His Glu Ser Lys Phe Tyr Gly Val Asn Phe Pro Ala Asp Gly Pro		
	125	130	135
	Val Met Lys Lys Met Thr Asp Asn Trp Glu Pro Ser Cys Glu Lys		
	140	145	150
25	Ile Ile Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser		
	155	160	165
	Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg Leu Arg Cys Gln Phe		
	170	175	180
	Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Arg Lys Met Pro Asp		
30	185	190	195
	Trp His Phe Ile Gln His Lys Leu Thr Arg Glu Asp Arg Ser Asp		
	200	205	210
	Ala Lys Asn Gln Lys Trp His Leu Thr Glu His Ala Ile Ala Ser		
	215	220	225
35	Gly Ser Ala Leu Pro		
	230		

	<210>	58	
	<211>	230	
	<212>	PRT	
5	<213>	zoanthus sp.	
	<220>		
	<223>	amino acid sequence of zFP538	
	<400>	58	
	Met Ala His Ser Lys His Gly Leu Lys Glu Glu Met Thr Met Lys		
10	5	10	15
	Tyr His Met Glu Gly Cys Val Asn Gly His Lys Phe Val Ile Thr		
	20	25	30
	Gly Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Thr Ile Asn		
	35	40	45
15	Leu Cys Val Ile Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile		
	50	55	60
	Leu Ser Ala Gly Phe Lys Tyr Gly Asp Arg Ile Phe Thr Glu Tyr		
	65	70	75
	Pro Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly		
20	80	85	90
	Tyr Thr Trp Gly Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile		
	95	100	105
	Cys Asn Val Asp Ile Thr Val Ser Val Lys Glu Asn Cys Ile Tyr		
	110	115	120
25	His Lys Ser Ile Phe Asn Gly Met Asn Phe Pro Ala Asp Gly Pro		
	125	130	135
	Val Met Lys Lys Met Thr Thr Asn Trp Glu Ala Ser Cys Glu Lys		
	140	145	150
	Ile Met Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser		
30	155	160	165
	Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg Tyr Arg Cys Gln Phe		
	170	175	180
	Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Ser Lys Met Pro Glu		
	185	190	195
35	Trp His Phe Ile Gln His Lys Leu Leu Arg Glu Asp Arg Ser Asp		
	200	205	210
	Ala Lys Asn Gln Lys Trp Gln Leu Thr Glu His Ala Ile Ala Phe		
	215	220	225

Pro Ser Ala Leu Ala

230

5	<210>	59	
	<211>	232	
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	<213>	<i>Discosoma striata</i>	
	<220>		
10	<223>	amino acid sequence of dsFP483	
	<400>	59	
	Met Ser Cys Ser Lys Ser Val Ile Lys Glu Glu Met Leu Ile Asp		
	5	10	15
	Leu His Leu Glu Gly Thr Phe Asn Gly His Tyr Phe Glu Ile Lys		
15	20	25	30
	Gly Lys Gly Lys Gly Gln Pro Asn Glu Gly Thr Asn Thr Val Thr		
	35	40	45
	Leu Glu Val Thr Lys Gly Gly Pro Leu Pro Phe Gly Trp His Ile		
	50	55	60
20	Leu Cys Pro Gln Phe Gln Tyr Gly Asn Lys Ala Phe Val His His		
	65	70	75
	Pro Asp Asn Ile His Asp Tyr Leu Lys Leu Ser Phe Pro Glu Gly		
	80	85	90
	Tyr Thr Trp Glu Arg Ser Met His Phe Glu Asp Gly Gly Leu Cys		
25	95	100	105
	Cys Ile Thr Asn Asp Ile Ser Leu Thr Gly Asn Cys Phe Tyr Tyr		
	110	115	120
	Asp Ile Lys Phe Thr Gly Leu Asn Phe Pro Pro Asn Gly Pro Val		
	125	130	135
30	Val Gln Lys Lys Thr Thr Gly Trp Glu Pro Ser Thr Glu Arg Leu		
	140	145	150
	Tyr Pro Arg Asp Gly Val Leu Ile Gly Asp Ile His His Ala Leu		
	155	160	165
	Thr Val Glu Gly Gly His Tyr Ala Cys Asp Ile Lys Thr Val		
35	170	175	180
	Tyr Arg Ala Lys Lys Ala Ala Leu Lys Met Pro Gly Tyr His Tyr		
	185	190	195

	Val Asp Thr Lys Leu Val Ile Trp Asn Asn Asp Lys Glu Phe Met		
	200	205	210
	Lys Val Glu Glu His Glu Ile Ala Val Ala Arg His His Pro Phe		
	215	220	225
5	Tyr Glu Pro Lys Lys Asp Lys		
	230		
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10	<212>	PRT	
	<213>	<i>Discosoma sp. "red"</i>	
	<220>		
	<223>	amino acid sequence of drFP583	
	<400>	60	
15	Met Arg Ser Ser Lys Asn Val Ile Lys Glu Phe Met Arg Phe Lys		
	5	10	15
	Val Arg Met Glu Gly Thr Val Asn Gly His Glu Phe Glu Ile Glu		
	20	25	30
	Gly Glu Gly Glu Gly Arg Pro Tyr Glu Gly His Asn Thr Val Lys		
20	35	40	45
	Leu Lys Val Thr Lys Gly Pro Leu Pro Phe Ala Trp Asp Ile		
	50	55	60
	Leu Ser Pro Gln Phe Gln Tyr Gly Ser Lys Val Tyr Val Lys His		
	65	70	75
25	Pro Ala Asp Ile Pro Asp Tyr Lys Lys Leu Ser Phe Pro Glu Gly		
	80	85	90
	Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp Gly Gly Val Val		
	95	100	105
	Thr Val Thr Gln Asp Ser Ser Leu Gln Asp Gly Cys Phe Ile Tyr		
30	110	115	120
	Lys Val Lys Phe Ile Gly Val Asn Phe Pro Ser Asp Gly Pro Val		
	125	130	135
	Met Gln Lys Lys Thr Met Gly Trp Glu Ala Ser Thr Glu Arg Leu		
	140	145	150
35	Tyr Pro Arg Asp Gly Val Leu Lys Gly Glu Ile His Lys Ala Leu		
	155	160	165
	Lys Leu Lys Asp Gly Gly His Tyr Leu Val Glu Phe Lys Ser Ile		
	170	175	180

Tyr	Met	Ala	Lys	Lys	Pro	Val	Gln	Leu	Pro	Gly	Tyr	Tyr	Tyr	Val	
					185				190					195	
Asp	Ser	Lys	Leu	Asp	Ile	Thr	Ser	His	Asn	Glu	Asp	Tyr	Thr	Ile	
					200				205					210	
5	Val	Glu	Gln	Tyr	Glu	Arg	Thr	Glu	Gly	Arg	His	His	Leu	Phe	Leu
					215				220					225	
	<210>														61
	<211>														232
10	<212>														PRT
	<213>														<i>Anemonia sulcata</i>
	<220>														
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	<400>														61
15	Met	Ala	Ser	Phe	Leu	Lys	Lys	Thr	Met	Pro	Phe	Lys	Thr	Thr	Ile
					5				10						15
	Glu	Gly	Thr	Val	Asn	Gly	His	Tyr	Phe	Lys	Cys	Thr	Gly	Lys	Gly
					20				25						30
	Glu	Gly	Asn	Pro	Phe	Glu	Gly	Thr	Gln	Glu	Met	Lys	Ile	Glu	Val
20					35				40						45
	Ile	Glu	Gly	Pro	Leu	Pro	Phe	Ala	Phe	His	Ile	Leu	Ser	Thr	
					50				55						60
	Ser	Cys	Met	Tyr	Gly	Ser	Lys	Thr	Phe	Ile	Lys	Tyr	Val	Ser	Gly
					65				70						75
25	Ile	Pro	Asp	Tyr	Phe	Lys	Gln	Ser	Phe	Pro	Glu	Gly	Phe	Thr	Trp
					80				85						90
	Glu	Arg	Thr	Thr	Tyr	Glu	Asp	Gly	Gly	Phe	Leu	Thr	Ala	His	
					95				100						105
	Gln	Asp	Thr	Ser	Leu	Asp	Gly	Asp	Cys	Leu	Val	Tyr	Lys	Val	Lys
30					110				115						120
	Ile	Leu	Gly	Asn	Asn	Phe	Pro	Ala	Asp	Gly	Pro	Val	Met	Gln	Asn
						125				130					135
	Lys	Ala	Gly	Arg	Trp	Glu	Pro	Ala	Thr	Glu	Ile	Val	Tyr	Glu	Val
						140				145					150
35	Asp	Gly	Val	Leu	Arg	Gly	Gln	Ser	Leu	Met	Ala	Leu	Lys	Cys	Pro
						155				160					165
	Gly	Gly	Arg	His	Leu	Thr	Cys	His	Leu	His	Thr	Thr	Tyr	Arg	Ser
						170				175					180

Lys Lys Pro Ala Ser Ala Leu Lys Met Pro Gly Phe His Phe Glu
 185 190 195
 Asp His Arg Ile Glu Ile Met Glu Glu Val Glu Lys Gly Lys Cys
 200 205 210
 5 Tyr Lys Gln Tyr Glu Ala Ala Val Gly Arg Tyr Cys Asp Ala Ala
 215 220 225
 Pro Ser Lys Leu Gly His Asn
 230

10 <210> 62
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 <212> PRT
 <213> *Discosoma sp. "green"*
 <220>
 15 <223> amino acid sequence of dgFP512
 <400> 62

Met Ser Ala Leu Lys Glu Glu Met Lys Ile Asn Leu Thr Met Glu
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 Gly Val Val Asn Gly Leu Pro Phe Lys Ile Arg Gly Asp Gly Lys
 20 20 25 30
 Gly Lys Pro Tyr Gln Gly Ser Gln Glu Leu Thr Leu Thr Val Val
 35 40 45
 Lys Gly Gly Pro Leu Pro Phe Ser Tyr Asp Ile Leu Thr Thr Met
 50 55 60
 25 Phe Gln Tyr Gly Asn Arg Ala Phe Val Asn Tyr Pro Glu Asp Ile
 65 70 75
 Pro Asp Ile Phe Lys Gln Thr Cys Ser Gly Pro Asn Gly Gly Tyr
 80 85 90
 Ser Trp Gln Arg Thr Met Thr Tyr Glu Asp Gly Gly Val Cys Thr
 30 95 100 105
 Ala Thr Ser Asn Ile Ser Val Val Gly Asp Thr Phe Asn Tyr Asp
 110 115 120

	Ile His Phe Met Gly Ala Asn Phe Pro Leu Asp Gly Pro Val Met		
	125	130	135
	Gln Lys Arg Thr Met Lys Trp Glu Pro Ser Thr Glu Ile Met Phe		
	140	145	150
5	Glu Arg Asp Gly Met Leu Arg Gly Asp Ile Ala Met Ser Leu Leu		
	155	160	165
	Leu Lys Gly Gly His Tyr Arg Cys Asp Phe Glu Thr Ile Tyr		
	170	175	180
	Lys Pro Asn Lys Val Val Lys Met Pro Asp Tyr His Phe Val Asp		
10	185	190	195
	His Cys Ile Glu Ile Thr Ser Gln Gln Asp Tyr Tyr Asn Val Val		
	200	205	210
	Glu Leu Thr Glu Val Ala Glu Ala Arg Tyr Ser Ser Leu Glu Lys		
	215	220	225
15	Ile Gly Lys Ser Lys Ala		
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	<213> <i>Discosoma sp. "magenta"</i>		
	<220>		
	<223> amino acid sequence of dmFP592		
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	Val Arg Met Glu Gly Thr Val Asn Gly His Glu Phe Glu Ile Lys		
	20	25	30
	Gly Glu Gly Glu Gly Arg Pro Tyr Glu Gly His Cys Ser Val Lys		
30	35	40	45

Leu Met Val Thr Lys Gly Gly Pro Leu Pro Phe Ala Phe Asp Ile
 50 55 60
 Leu Ser Pro Gln Phe Gln Tyr Gly Ser Lys Val Tyr Val Lys His
 65 70 75
 5 Pro Ala Asp Ile Pro Asp Tyr Lys Lys Leu Ser Phe Pro Glu Gly
 80 85 90
 Phe Lys Trp Glu Arg Val Met Asn Phe Glu Asp Gly Gly Val Val
 100 105 110
 Thr Val Ser Gln Asp Ser Ser Leu Lys Asp Gly Cys Phe Ile Tyr
 10 115 120 125
 Glu Val Lys Phe Ile Gly Val Asn Phe Pro Ser Asp Gly Pro Val
 130 135 140
 Met Gln Arg Arg Thr Arg Gly Trp Glu Ala Ser Ser Glu Arg Leu
 145 150 155
 15 Tyr Pro Arg Asp Gly Val Leu Lys Gly Asp Ile His Met Ala Leu
 160 165 170
 Arg Leu Glu Gly Gly His Tyr Leu Val Glu Phe Lys Ser Ile
 175 180 185
 Tyr Met Val Lys Lys Pro Ser Val Gln Leu Pro Gly Tyr Tyr Tyr
 20 190 195 200
 Val Asp Ser Lys Leu Asp Met Thr Ser His Asn Glu Asp Tyr Thr
 205 210 215
 Val Val Glu Gln Tyr Glu Lys Thr Gln Gly Arg His His Pro Phe
 220 225 230
 25 Ile Lys Pro Leu Gln
 235

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/29405

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/68; C07K 14/435

US CL :435/6, 69.1; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 968; 530/350; 424/9.6, 436/172

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
***	The sequence diskette submitted with the description was defective; thus the references listed below were obtained solely by a WORD search, and not by a search of the SEQ ID NOs.	***
X, P	MATZ et al. Fluorescent proteins from nonbioluminescent Anthozoa species. Nature Biotechnology. October 1999, Volume 17, No. 10, pages 969-973, entire document.	1-10
X, P	DE 197 18 640 A1 (WIEDENMANN) 22 July 1999, entire document.	3-10

Further documents are listed in the continuation of Box C.

See patent family annex.

• Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 FEBRUARY 2000

Date of mailing of the international search report

02 MAR 2000

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